The two organisms are not identical. The rejection should be withdrawn.

Judicially-Created Doctrine for Obviousness-Type Double-Patenting

Claims 1-18 and 33 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 1-16 of US 5686276, and provisionally over USSN 08/687,852. Claims 1, 3-5, 7-19 and 32 and 33 have been canceled from this application. Only the remaining Claims 2 and 6 are subject to this rejection.

Claims 2 and 6 are patentably distinct from the cited claims of either US 5686276 or USSN 08/687,852. Claim 2, as now amended, is drawn to a process for the production of 1,3-propanediol from glycerol, using recombinant fungal and bacterial species transformed with a gene encoding a glycerol dehydratase enzyme. Claim 6 is drawn to a process for the production of 1,3-propanediol from a carbon substrate using an organism containing *dhaT* and a gene expressing an active dehydratase enzyme.

Claims 1-16 of US 5686276 expressly exclude glycerol as a carbon substrate starting material for the production of 1,3-propanediol. The cited claims of USSN 08/687,852 are limited to a process using a gene encoding a diol dehydratase enzyme. Neither US 5686276 nor USSN 08/687,852 require the presence of *dhaT*. Because Claims 2 and 6 contain subject matter that is patentably distinct from either the claims of US 5686276 or USSN 08/687,852, Applicants respectfully request the removal of this rejection.

Claims 20-21, 23-32 are rejected under the judicially crated doctrine of obviousness-type double patenting as being unpatentable over Claims 1-10 of US 5633362. The Examiner argues that Claims 20-21, 23-32 of the instant case differ merely in scope from Claims 1-10 of US 5633362 and are drawn to genes on the same cosmid. The rejection should be withdrawn because the subject matter of Claims 20-21, and 23-32 is

patentably distinct from that of Claims 1-10 of U.S. 5,633,362 as the cosmids contain different genes and are functionally different.

Claims 20-21, 23-32 are drawn to the cosmid pKP1 and an *E. coli* transformed with the cosmid, the transformed cell having glycerol dehydratase activity (see page 6, lines 11-16 of the specification). The cosmid was isolated by a process that selected for the ability of transformants to convert glycerol to 1,3-propanediol and contained portions of the glycerol dehydratase gene as revealed by sequence comparisons (see page 10, lines 34-35 and page 11, lines 1-2). A glycerol dehydratase enzyme is defined as a dehydratase having a preferred substrate of glycerol (see page 9, lines 17-21).

Claims 1-10 of US 5633362 are drawn to the cosmid pKP4 and an *E. coli* transformed with the cosmid, having diol dehydratase activity. The cosmid pKP4 contains the diol dehydratase gene as revealed by sequencing and homology comparisons (see page 11, lines 1-5). A diol dehydratase is defined as a dehydratase having preferred substrate of 1,2-propanediol (see page 11, lines 1-5).

Because the subject matter of the instant Claims 20-21, 23-32 and Claims 1-10 of US 5633362 are drawn to cosmids and transformed cells containing different genes and different functionalities, the subject matter of these two sets of claims is patentably distinct. Accordingly, Applicants request the removal of the rejection under the judicially created doctrine of obviousness-type double patenting.

Enablement Under 35 USC §112, first paragraph

The Examiner rejects Claims 1-12, 14-18 and 31-33 under 35 USC §112, first paragraph, finding the specification is non-enabling for the production of 1,3-propanediol by any microorganism transformed with any diol dehydratase from any organism. Claims 1, 3-5, 7-19 and 32 and 33 are canceled. Claims 2, 6, and 31 still fall under this rejection.

The Examiner argues that the specification is enabling only for the isolation and manipulation of the dehydratase gene from *Klebsiella*, and is not generally enabling for any organism. Claim 2, as now amended, is drawn to a process to the product of 1,3-propanediol from glycerol, using recombinant fungal and bacterial species transformed with a gene encoding a glycerol dehydratase enzyme. Claim 6 is drawn to a process for the product of 1,3-propanediol from a carbon substrate using an organism containing *dhaT* and a gene expressing an active dehydratase enzyme. Claim 31 has been amended in scope to encompass recombinant fungal microorganisms expressing either a glycerol dehydratase or a diol dehydratase enzyme. Claim 32 is canceled, and its subject matter has been incorporated into the scope of Claim 31.

The specification is enabling for the claims as now amended. Although the examples contain only a dehydratase enzyme isolated from *Klebsiella*, pages 9-11 of the specification teach methods for cosmid and gene isolation and reference is given to other bacteria that are known to contain dehydratase activity (page 11, line 8). It is well within the skill of the routineer to apply the teaching of the specification, in combination with the detailed examples, to isolate other genes encoding a dehyratase enzyme commensurate with the instant invention.

Additionally, there is adequate teaching in the examples to support the scope of the claims as now amended with respect to the use of fungal host cells. Example 7 details the construction of a recombinant *Pichia;* Examples 9-11 teach the construction of recombinant *Saccharomyces;* and Examples 22-23 detail a recombinant *Aspergillus*. Each of these recombinants expresses an active dehydratase enzyme. The skilled artisan would be able to use the teaching of these examples to use fungal microorganisms as hosts for an appropriate gene encoding either a glycerol or diol dehydratase enzyme without undue experimentation.

Claims 26-30 are rejected under 35 USC §112, first paragraph, for containing subject matter that is not enabled by the specification.

Applicants enclose a declaration attesting to deposit of the claimed microorganisms under the terms of the Budapest Treaty as requested. The filing of this declaration is not an admission as to the necessity of this declaration to satisfy the enablement requirements of the statute. Withdrawal of the rejection is requested.

Definiteness of the Specification Under 35 USC §112, second paragraph

Claims 1, 2, 5, 6, 12 and 14 are rejected under 35 USC §112, second paragraph for indefiniteness. All but Claims 2 and 6 of this grouping have been canceled.

Claim 2 has been amended to remove language describing the latent capability of gene expression.

Claim 6 has been rewritten in independent form and amended to clarify the open reading frames required in the claimed process.

The claims as now amended comply with 35 USC §112, second paragraph. Applicants respectfully request the removal of this rejection.

Novelty Under 35 USC 102(b)

Claims 1, 3-10, 13, 19-21, 23 and 33 are rejected under 35 USC §102(b) as anticipated by Tong et al. Claims 1, 3-10, 13, 19 and 33 are canceled.

Claims 20 and 21 are drawn to the cosmid containing a glycerol dehydratase gene isolated from *K. pneumoniae* and organisms containing the cosmid respectively. Claim 23 is drawn to an organism containing the cosmid from *K. pneumoniae* having the glycerol dehydratase gene and any other gene encoding a protein having a biological function.

Tong et al. (*Appl. Biochem. Biotechnol.* 34-35 (1992) hereinafter Tong-1992) teach the expression of the *Klebsiella*

dha regulon in *E. coli* for the conversion of glycerol to 1,3-Propanediol. Referenced in Tong-1992 is Tong et al., *App. Enivr. Microbiol.*, 57, 3541, (1991); (hereinafter Tong-1991, copy enclosed), teaching the isolation of the cosmid used in Tong-1992 that contains a gene encoding a dehydratase activity from *Klebsiella* and the transformation of *E. coli* with the cosmid for the conversion of glycerol to 1,3-Propanediol. Neither Tong-1991 nor Tong-1992 teach the conversion of non-glycerol carbon substrates to 1,3-Propanediol.

A valid rejection under 35 USC §102(b) requires that each and every element of the rejected claims be described in the cited reference. The instant claims differ from the description of the cited reference in that the instant cosmid is physically different from that taught by Tong-1992 and Tong-1991, both in size and in the open reading frames identified.

The instant cosmid is an insert of no more than 35kb (see page 4, line 36; page 22, line 12). In contrast, the insert in the cosmid of Tong-1991 is merely 18.2 kb (page 3544, column 1). Further, Tong-1991 describe only that pTC1 includes the four enzymes 1,3-propanediol oxidoreductase (known as *dhaT*) the glycerol dehydrogenase (known as *dhaD*) the DHA kinase (known as *dhaK*) and glycerol dehydratase (known as *dhaB1*, B2, and B3) (Tong-1991; page 3545, column 1). In contrast, Applicants invention includes the additional open reading frames *dhaR* and *dhaX* (see page 24, lines 1-12 of the specification).

Furthermore, neither Tong reference suggests that another carbon substrate can be used in the place of glycerol. Tong-1992 presents data showing that organisms containing the glycerol dehydratase gene and enzyme are unable to produce 1,3-Propanediol in the presence of only glucose (page 152, Table 1). Tong-1992 observes 1,3-propanediol production when glycerol is used as the sole carbon source, and increased conversion of exogenously added glycerol to 1,3-propanediol when a sugar is also present. However, in no case is 1,3-propanediol observed in the absence of exogenously added

glycerol. Tong-1992 do not suggest that any of the 1,3-propanediol observed in their glycerol/sugar cofermentation experiments is derived from sugar carbon. Instead, they argue that reducing equivalents (hydrogen) derived from sugar can be used to drive the carbon yield of 1,3-propanediol from glycerol towards its theoretical maximum of 100%.

Thus, as the cosmid of Tong-1991 and Tong-1992 is clearly physically different from the instant cosmid, the subject matter of the instant claims is not anticipated by either reference. In view of the foregoing, Applicants respectfully request that the rejection under 35 USC 102(b) be removed.

Claim 19 is rejected under 35 USC §102(b) as being anticipated by Walborsky et al. Claim 19 is canceled.

Claims 31-32 are rejected under 35 USC §102(b) as being anticipated by Weinstock et al. The Examiner argues that Weinstock et al. is anticipatory because it provides the expression of the homolog of HIS3 (encoding IGPH) of *S. kluverii* in *S. cerevisiae*.

Claim 31 has been amended in scope to recombinant yeast or fungal microorganisms expressing a bacterial gene encoding a dehydratase enzyme. The specification defines dehydratase enzyme as "any enzyme that is capable of isomerizing or converting a glycerol molecule to the product 3-hydroxypropion-aldehyde. For the purposes of the present invention the dehydratase enzymes include a glycerol dehydratase and a diol dehydratase having preferred substrates of glycerol and 1,2-propanediol, respectively." (page 9, line 20).

Weinstock et al. describe the cloning of the HIS3 gene homolog in yeast, encoding imidazole glycerol phosphate dehydratase (IGPD). IGPD catalyzes a dehydration reaction of imidazoleglycerol phosphate (ICP) to form imidazoleacetol phosphate in the biosynthesis of histidine (Ohta et al., *Weed Sci.* (1997), 45(5), 610-620, copy enclosed). Thus, IGPD is not

within the scope of "dehydratase enzyme" as defined in the instant specification. As each and every element of the instant claim is not embodied in or obvious from the cited reference, Applicants respectfully request the removal of this rejection under 35 USC §102(b).

In view of the foregoing, allowance of the above-referenced application is respectfully requested. Should any matter remain unresolved, please contact the undersigned.

Respectfully submitted,

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